TPLGPASSLPQSFLLKCLEQVRKIQGDGAALQEKLCATYKLCHPEELVLLGHSLGIPWA PLSSCPSQALQLAGCLSQLHSGLFLYQGLLQALEGISPELGPTLDTLQLDVADFATTIW QQMEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLEVSYRVLRHLAQP

hGCSF wild type

ATGACTCCATTAGGTCCAGCTTCCTCTGCCGCAAAGCTTCCTGCTGAAATGCCTGGAACAGGTTCGTAAAATCCAGGGTGATGG TGCTGCTCTGCAGGAAAAACTGTGCGCTACCTACAAACTGTGCCATCCGGAAGAACTGGTTCTGCTGGGTCACTCCCTGGGTATCC CAGGGTCTGCTGCAAGCTCTGGAAGGTATCTCCCCGGAACTGGGTCCGACCCTGGACACTCTGCAGCTGGACGTCGCTGACTTCGC TACCACCATCTGGCAGCAGATGGAAGAACTGGGTATGGCTCCGGCTCTGCAGCCGACCCAGGGTGCTATGCCGGCTTTCGCTTCCG CTTTCCAGCGTCGCGCAGGTGGCGTTCTGGTTGCTAGCCACCTGCAGGTTCCTGGAAGTTTCCTACCGTGTTCTGCGTCACCTG GCTCAGCCGTGA

FIG.

| core4     | core4v  | bndry4_2  | dry4_core   | bndry4_AD  | Jry4_AD_(  |   |
|-----------|---|---|---|--|--|---|
|           |   |   |   |  | core4  |   |
| 21<br>151 | 21<br>153   | 14<br>120   | 14<br>120   | 14   | 14   |   |
| 24<br>152 | 24<br>154   | 20<br>145   | 20<br>145   | 2.0  | 20   |   |
| 28<br>153 | 28<br>157   | 27  | 146   | 27   | 27   |   |
| 31<br>154 | 31<br>160   | 32<br>147   | 32<br>147   | 32   | 32   |   |
| 75<br>157 | 75<br>161   | 34<br>148   | 34<br>148   | 34   | 34   |   |
| 78<br>160 | 78<br>167   | 38<br>155   | 38<br>155   | 38   | 38   |   |
| 82        | 82<br>168   | 77<br>156   | 77  | 145  | 145  |   |
| 85<br>168 | 8<br>5  | 79<br>164   | 79  | 146  | 146  |   |
| 89        | 68  | 84<br>170   | 84<br>170   | 147  | 147  |   |
| 103       | 103   | 91  | 91  | 148  | 148  |   |
| 106       | 106   | <u>ი</u>  | <b>6</b>  | 155  | 155  |   |
| 110       | 110   | 102   | 102   | 156  | 156  |   |
| 113       | 113   | 107   | 107   | 164  | 164  |   |
| 114       | 114   | 109   | 109   | 170  | 170  |   |
| 117       | 117   | 116   | 116   |  |  |   |
| 149       | 151   |   |   |  |  | `   |
|           | 17 21 24 28 31 75 78 82 85 89 103 106 110 113 114 117 14<br>150 151 152 153 154 157 160 161 168 | 17     21     24     28     31     75     78     82     85     89     103     106     110     113     114     117     14       150     151     152     153     154     157     160     161     163     103     106     110     113     114     117     15       152     153     154     157     160     161     167     168     82     85     89     103     106     110     113     114     117     15 | 17       21       24       28       31       75       78       82       85       89       103       106       110       113       114       117       14         150       151       152       153       154       157       160       161       167       168       85       89       103       106       110       113       114       117       15         152       153       154       157       160       161       167       168       84       91       99       102       107       109       116         1-2       145       146       147       148       155       156       164       170       99       102       107       109       116 | 17 21 24 28 31 75 78 82 85 89 103 106 110 113 114 117 14 150 151 152 153 154 157 160 161 168 82 85 89 103 106 110 113 114 117 15 152 153 154 157 160 161 167 168 152 153 154 157 160 161 167 168 152 153 154 157 160 161 167 168 154 170 199 102 107 109 116 155 156 145 146 147 148 155 156 164 170 156 164 170 199 102 107 109 116 | 150 151 152 153 154 157 160 161 168 85 89 103 106 110 113 114 117 14  152 153 154 157 160 161 167 168 85 89 103 106 110 113 114 117 15  152 153 154 157 160 161 167 168 82 85 89 103 106 110 113 114 117 15  120 145 146 147 148 155 156 164 170 99 102 107 109 116  1AD 14 20 27 32 34 38 77 79 84 91 99 102 107 109 116  1AD 14 20 27 32 34 38 145 146 170 199 102 107 109 116 | 150 151 152 153 154 157 160 161 168 89 103 106 110 113 114 117 14  150 151 152 153 154 157 160 161 168 85 89 103 106 110 113 114 117 15  151 152 153 154 157 160 161 167 168 82 85 89 103 106 110 113 114 117 15  152 153 154 157 160 161 167 168 82 85 89 103 106 110 113 114 117 15  152 153 154 157 160 161 167 168 156 164 170  150 145 146 147 148 155 156 164 170  150 150 107 109 116  150 150 150 150 150 150 150 150 150 150 |

F/G.\_2

G-CSF Designs - Optimal Sequences Selected by PDA\*

| t<br>_2<br>_cor<br>_AD<br>_AD<br>_AD     | MTPLGPASSLPQS         | FLL                   | KCLE        | OVERT            | 100                 |                       |                  | Subbe       | T,VI,T,C    | LGHSLGIPWA            | Q VIV       |
|--|-----------------------|-----------------------|-------------|------------------|---------------------|-----------------------|------------------|-------------|-------------|-----------------------|-------------|
| AD AD AT A A D A D A D A D A D A D A D A |                       |                       |             | X + 1 1 1 1 X    | JOIN GAL            | ALQEKLCA              | ATYKL            | ממענו       | 1           |                       | 77770       |
|  |                       |                       |             | L                | 田                   | Н                     | ĸ                |             |             |                       |             |
| _AD                                      | 34                    |                       | H           | Ţ                | EA                  | 디                     | н                |             |             |                       |             |
| _ADV16                                   |                       | H                     |             | E E              | 曰                   | H                     | Ħ                |             |             |                       |             |
| core4<br>core4_V1677                     | _core4                |                       | ч           | L                | EA                  | 디                     | H                |             |             |                       |             |
| core4_V1671                              |                       |                       | н           |                  | K                   |                       |                  |             |             |                       |             |
| Gores                                    | <b>~</b>              |                       | н           | Н                | ď                   |                       |                  |             |             |                       |             |
|  |                       |                       | H           |                  | 4                   | Н                     |                  |             |             |                       |             |
| sm0                                      | !<br>!<br>!<br>!      | 1<br>1<br>1<br>1<br>1 |             | 1 1 1 1 1 1 1    | - A                 | 1<br>1<br>1<br>1<br>1 | !<br>!<br>!<br>! | 1<br>1<br>1 | !<br>!<br>! | [<br>]<br>]<br>[<br>] | i<br>1<br>1 |
| £m2                                      |                       |                       | ď           |                  | ď                   |                       |                  |             |             |                       |             |
| £m3                                      |                       |                       | ы           |                  | Ø                   |                       |                  |             |             |                       |             |
| £m4                                      |                       |                       | н           |                  | æ                   |                       |                  |             |             |                       |             |
| £m7                                      |                       |                       | ı           |                  | ď                   |                       |                  |             |             |                       |             |
|  | .,,                   |                       |             |                  |                     |                       |                  |             |             |                       |             |
|  | 70                    | 80                    |             | 90               |                     | 100                   |                  | 110         |             | 120                   |             |
| Ö  | PSQALQLAGCL           | SQLHSGLF              | FLYQ        | GLLQAL           | EGISE               | PELGPTL               | DTLQLI           | LDVADFAT    | Ę           | 1                     |             |
| bndry4_2                                 |                       | ᄓ                     |             | X                |                     | ı<br>∧                |                  | E-)         | Н           | ļ rā                  |             |
| ndry4_core4                              |                       | Ţ <b>Ŀ</b>            | Ŀų          | ×                |                     | ΚV                    | Н                | EI L        | Н           | ı                     |             |
| ndry4_AD                                 |                       |                       |             |                  |                     |                       |                  |             |             |                       |             |
| bndry4_AD_core4                          |                       | Ľι                    | Įτι         |                  |                     | >                     |                  | H           |             |                       |             |
| ore4                                     |                       | ᄕ                     | ഥ           |                  |                     | >                     |                  | П           |             |                       |             |
| ore4_V167A                               |                       | ഥ                     | Ĺτι         |                  |                     |                       |                  | н           |             |                       |             |
| 2re3                                     | 1                     | E4                    | F4          | Įτί              |                     |                       |                  | H           |             |                       |             |
| sm0                                      | t<br>1<br>1<br>1<br>1 | !<br>!<br>!<br>!      | !<br>{<br>! | 1<br>t<br>t<br>t | <br> <br> <br> <br> | <br>                  | 1<br>1<br>1<br>1 | 1 1         | <br>        | 1<br>1<br>1<br>1      | 1<br>1<br>1 |
| . 2                                      |                       |                       |             |                  |                     |                       |                  |             |             |                       |             |
| fm3                                      |                       | ᄕᅫ                    |             |                  |                     |                       |                  | H           |             |                       |             |
| fm4                                      |                       | Ē                     |             |                  |                     |                       |                  | H           |             |                       |             |
| £m7                                      |                       | ſ×ι                   | Ĺτι         |                  |                     |                       |                  | H           |             |                       |             |

FIG.\_3A

\_\_

|                 | 130   | 130 140 150 160 170 |        | 150 |           | 16               | 0    | 170                                  |             |
|-----------------|---|---------------------|--------|-----|-----------|------------------|------|--------------------------------------|-------------|
| hGCSFwt ME      | $\tt MEELGMAPALQPTQGAMPAFASAFQRRAGG{\bf VL}{\bf V} ASHLQSFLEVSYRV{\bf L}RHLAQP$ | AMPAFASAF           | QRRAG  | GVL | VASHI     | QSFL             | EVSY | RV <b>L</b> RHLAQP                   |             |
| bndry4_2        |   |                     | KED    |     | II        |                  | Ø    |                                      |             |
| bndry4_core4    |   |                     | KED    | н   | I         |                  | Ø    | ᄕᅭ                                   |             |
| bndry4_AD       |   |                     | KET    |     | II        |                  | Ø    |                                      |             |
| bndry4_AD_core4 | e4.   |                     | KED    | н   | III       |                  | Ø    | ᄕ                                    |             |
| core4           |   |                     |        | н   | ш         |                  |      | ᄕᅭ                                   |             |
| core4_V167A     |   |                     |        | н   |           | I WF             |      | AF                                   |             |
| core3           |   |                     |        | н   | u         |                  |      | ᄕ                                    |             |
| sm0             | 1<br>f<br>f<br>t<br>l<br>l<br>l<br>t  | !<br>!<br>!<br>!    | !<br>! | 1   | 1 1 1 1 1 | !<br>!<br>!<br>! | <br> | 1<br>1<br>1<br>1<br>4<br>1<br>8<br>1 | 1 1 1 1 1 1 |
| fm2             |   |                     |        | н   | <b>.</b>  |                  |      |                                      |             |
| fm3             |   |                     |        |     |           |                  |      | ĺΨ                                   |             |
| £m4             |   |                     |        | н   | ш         |                  |      | Ē                                    |             |
| £m7             |   |                     |        | Н   | _         |                  |      | ſΞŧ                                  |             |

Core4 mutant ground state; Monte Carlo analysis shows the ground state with Phe instead of Trp for \*Sequences shown below dotted lines were not obtained from PDA calculations but were The sequence selected for Core4\_V167A is not the derived by reverting some core4 or core3 mutant positions to wild type. position 160, and Leu instead of Phe for position 161 (see Table 4) positions are indicated in bold.

FIG.\_3B

FIG.\_4

| Core4 - Monte Carlo Analysis - Ground State and Allowed Amino Acids and Their Number of Occurrences (For the Top 1000 Sequences) |                 |   |
|--|-----------------|---|
| state<br>rthe1   |                 | 313   |
| ound S   |                 | <u> </u>  |
| <b>s</b> - Gro<br>urrence  |                 | 229<br>287<br>961<br>172<br>707<br>707<br>707<br>140<br>321<br>264<br>143<br>143<br>187<br>187<br>161 |
| <b>Analysi</b><br>er of Occu   | _               |   |
| <b>te Carlo</b><br>Numbe   | ind<br>ite      | 736<br>736<br>747<br>747<br>747<br>747<br>747<br>747<br>747<br>747<br>747<br>74                       |
| Mont<br>Id Their   | Ground<br>State | LEU ARAL LEU RAL LEU LEU LEU LEU LEU LEU LEU LEU LEU LE   |
| Core4  | Position        | 7   |
|  | hG-CSF          | CYS<br>LEU SEL SEL LEU SEE<br>SEL SEL SEL SEL SEL SEL SEL SEL SEL SEL                                 |

\*position where Monte Carlo didn't find an alternative and where the top amino acid is the wild type  $\, extstyle\,$ 

Щ.

FIG.\_5

Coredv - Monte Carlo Analysis (Ground State and Allowed Amino Acids

| <b>Core4v - Monte Carlo Analysis</b> (Ground State and Allowed Amino Acids and Their Number of Occurrences (For the Top 1000 Sequences) | ·               |   |                   |                |            |            |            |                     |        |                |                  |            |            |     |
|---|-----------------|---|-------------------|----------------|------------|------------|------------|---------------------|--------|----------------|------------------|------------|------------|-----|
| Allowed<br>Sequen   |                 | 17                                      | 47                |                | 94         | 295        |            |                     |        |                |                  |            |            |     |
| ate and<br>p 1000   |                 | 吊                                       | =                 |                | ΤYΒ        | 当          |            |                     |        |                |                  |            |            |     |
| und Sta<br>the To   |                 | 251<br>300                              | 257               |                | 16         | 46<br>269  | 149        |                     |        |                |                  | 184        |            |     |
| s (Gro  |                 | 빌빌                                      | VAL               |                | LE<br>T    | LEU        | LEU        |                     |        |                |                  | 빌          |            |     |
| <b>ınalysi</b><br>urrence   |                 | 51<br>682<br>61                         | 193               | 7 + 7          | α <u>ç</u> | 78         | 54<br>405  |                     | 61     | :              | 414              | 18<br>448  | 156        |     |
| carlo ⊿<br>r of Occ   | _               | X & & & & & & & & & & & & & & & & & & & | LEU               | VAL            | VAL        | ALA        | VAL<br>VAL |                     | VAL    |                | VAL              | VAL<br>TRP | H<br>H     |     |
| <b>- Monte</b><br>r Numbe   | und<br>ite      | 697<br>682<br>938                       | 808<br>694<br>699 | 982<br>982     | 887        | 357        | 945<br>445 | 666                 | 800    | 0 0 1<br>0 0 0 | 282<br>666<br>67 | 797<br>551 | 843<br>999 | 666 |
| <b>ore4v</b><br>Id Theii  | Ground<br>State | LEU<br>VAL                              | ALA<br>LEU        | E<br>문<br>된    | 품.         | WAL<br>WAL |            | LEU                 | Ш<br>Ш |                | ILE<br>ALA       | HE         | LEU<br>ALA | 띺   |
| <b>Table 4.</b> Car   | Position        | 24<br>71<br>72<br>74                    | 28<br>31<br>75*   | 8 4 8<br>8 2 8 | 822        | 103        | 106<br>110 | ττ<br>εξ<br>4<br>13 | 117*   | 152            | 153<br>154*      | 157        | 161<br>167 | 168 |
| <u> </u>  | hG-CSF          | CYS<br>VAL<br>ILE                       | GEV<br>LEU<br>LEU | EE             | T -<br>R - | LEF        | LEU<br>VAL | PHE<br>ALA          |        | LEU            | VAL<br>ALA       | 먎          | LEU<br>VAL | LEO |
|   |                 |   |                   |                |            |            |            |                     |        |                |                  |            |            |     |

\*position where Monte Carlo didn't find an alternative and where the top amino acid is the wild type  $\, extcircles$ 

|        | Table 5. | Core;<br>and Tr | <b>3 - Mon</b> l<br>neir Num | t <b>e Carlo</b><br>iber of O | <b>Analy</b> s<br>courrei | <b>sis</b> (Gro<br>nces (F | or the | tate an<br>Top 10( | d Allow<br>30 Seq | red Amil<br>uences | o Acids  |  |
|--------|----------|-----------------|------------------------------|-------------------------------|---------------------------|----------------------------|--------|--------------------|-------------------|--------------------|----------|--|
| hG-CSF | Position | Ground<br>State | und<br>ate                   |                               |                           |                            |        |                    |                   |                    |          |  |
| CYS    | 17       | LEU             | 585                          | VAL                           | 35                        | 밀                          | 379    |                    |                   |                    |          |  |
| VAL    | 21       | VAL             | 551                          | ALA                           | र्फ                       | <u> </u>                   | 291    | DHE<br>BHE         | 141               | ΤΥΒ                | <b>-</b> |  |
| 빌      | 24       | <u>Н</u>        | 657                          | ALA                           | 31                        | VAL                        | 303    | LEC                | ω                 |                    |          |  |
| GLY    | 28       | ALA             | 928                          | LEU                           | 71                        |                            |        |                    |                   |                    |          |  |
| LEU    | 31       | LEU             | 888                          | VAL                           | 111                       |                            |        |                    |                   |                    |          |  |
| ΓΥS    | 35       | Щ               | 785                          | VAL                           | 214                       |                            |        |                    |                   |                    |          |  |
|        |          | i               | 1 1                          |                               |                           |                            |        |                    |                   |                    |          |  |

| $\blacksquare$                              |          |     |     |              |     |     |     |     |     |     |     |             |      |         |  |
|---|----------|-----|-----|--------------|-----|-----|-----|-----|-----|-----|-----|-------------|------|---------|--|
|   | 61       |     |     |              |     |     |     |     |     |     |     |             |      | _       |  |
|   | TYR      |     |     |              |     |     |     |     |     |     |     |             |      | TRP     |  |
|   | 75       |     |     |              |     |     |     |     |     |     |     |             |      | 50      |  |
|   | LE<br>LE |     |     |              |     |     |     |     |     |     |     |             |      | H       |  |
|   | 12       |     |     |              |     |     |     |     |     |     | 291 |             |      | Ŋ       |  |
|   | LEU      |     |     |              |     |     |     |     |     |     | Щ   |             |      | LEU     |  |
|   | 149      | 136 |     |              | #   |     |     |     |     |     | 294 | 44          |      | 168     |  |
|   | VAL      | 뮢   |     |              | TRP |     |     |     |     |     | LEU | PH<br>H     |      | VAL     |  |
| - 4<br>- 4                                  | 9        | 12  | 363 |              | 214 |     |     |     | 11  | 106 | 4   | <del></del> |      | 15      |  |
| AA<br>VAL                                   | ALA      | ALA | TRP |              | LEU |     |     |     | 빌   | VAL | ALA | ALA         |      | ALA     |  |
| 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0       |          | 851 | 636 | 666          | 674 | 666 | 666 | 666 | 888 | 893 | 400 | 954         | 666  | 790     |  |
|   | PHE      | LEU | 出   | LEU          | 띺   | 뮢   | 밀   | LEU | LEU | LEU | VAL | LEU         | ALA  | II<br>I |  |
| - 0 6 4 4 4 6 6 7 4 4 4 4 4 4 4 4 4 4 4 4 4 | 78       | 85  | 82  | *<br>88<br>8 | 68  | 95  | 35* | *66 | 103 | 106 | 110 | 113         | 114* | 117     |  |
|   | E i      | EU  | Ϋ́R | 品            | 교   | EU  | щ   | EU  | 凹   | EU  | ÄL  | 里           | ĻΑ   | щ       |  |

\_\_\_

Table 5. Core3 - Monte Carlo Analysis (Ground State and Allowed Amino Acids and Their Number of Occurrences (For the Top 1000 Sequences)

|             | ·               |  |
|-------------|-----------------|--|
|             |                 | 104  |
|             |                 | ILE 10<br>PHE 16                               |
| )<br>)<br>) |                 | 179  |
| ;<br>;<br>) |                 | VAL  |
|             |                 | 22<br>425<br>6                                 |
| 5           |                 | ALA<br>ALA                                     |
| 3           | und<br>ite      | 99999999999999999999999999999999999999         |
| 5           | Ground<br>State | PHE<br>LEU<br>PHE<br>PHE<br>PHE<br>PHE         |
| 3           | Position        | 151<br>152<br>153<br>154<br>160<br>160         |
|             | hG-CSF          | PHE VAL LEU LEU LEU LEU LEU LEU LEU LEU LEU LE |
|             |                 |  |

\*position where Monte Carlo didn't find an alternative and where the top amino acid is the wild type

Table 6. Bndrv4 2 - Monte Carlo Analysis (Ground State and Allowed Amino Acids

| lable 6. Bndry4_2 - Monte Carlo Analysis (Ground State and Allowed Amino Acids<br>and Their Number of Occurrences (For the Top 1000 Sequences) |                 |                         |  |                   |     |          |            |            |             |     |     |     |            |     |            |            |                       |     |        |
|--|-----------------|-------------------------|--|-------------------|-----|----------|------------|------------|-------------|-----|-----|-----|------------|-----|------------|------------|-----------------------|-----|--------|
| d Allow<br>Seque   |                 |                         |  | 154               |     |          |            |            |             |     |     |     |            |     |            |            |                       |     | 227    |
| tate and<br>p 1000   |                 |                         |  | ΓΧ                |     |          |            |            |             |     |     |     |            |     |            |            |                       |     | GLN    |
| und S<br>the To  |                 |                         | 73   | 237               |     |          |            |            |             |     | 52  |     |            |     |            | 330        |                       |     | 248    |
| <b>sis</b> (Gro  |                 |                         | GLU  | 0.00              |     |          |            |            |             |     | ΓXS |     |            |     |            | ASP        |                       |     | GLU    |
| <b>Analys</b><br>urrence   |                 |                         | 209  | 88                |     |          | 0          | 5.4<br>5.4 | -<br>-<br>- | 474 | 198 |     | 349        | 108 |            | 268        |                       |     | 11     |
| e Carlo  | _               |                         | Щ!   | <u> </u>          |     |          | <u>.</u>   |            | !           | VAL | LEU |     | OLU<br>GLU | GLN |            | ALA        |                       |     | LEU    |
| <b>2 - Mont</b><br>r Numbe   | around<br>State | & o o<br>o o o<br>o o o | 999<br>717   | 904<br>909<br>909 | 666 | 666      | 999        | 562        | 993         | 525 | 749 | 666 | 650        | 891 | 666        | 401        | n o                   | 666 | 380    |
| <b>dry4_</b><br>d Thei   | O               |                         | SES<br>CES   | SLN<br>GLN        | LEU | LEU      | Z Z        |            |             | GLU | Щ   | LEU | GLN        | ΓΥS | 015<br>101 | 는 <u>-</u> | _<br>_<br>_<br>_      | ALA | HSP    |
| <b>ble 6. Bn</b><br>an   | Position        | 44<br>20<br>70          | 3888   | 38<br>77*         | 79  | ****     | ი<br>ი     | 102        | 107         | 109 | 116 | 120 | 145        | 146 | 147        | 148<br>17  | 2<br>0<br>0<br>0<br>0 | 164 | 170    |
| <u>a</u>   | hG-CSF          | LEU<br>GLN              | SCN<br>SCN<br>FAS<br>FAS<br>FAS<br>FAS<br>FAS<br>FAS<br>FAS<br>FAS<br>FAS<br>FAS | GLN               | SH. | LEU<br>S | A <u>-</u> | 를<br>문     | GLN         | VAL | THR | Z I | GLN<br>GLN | ARG | ARG        | ALA<br>ED  | H<br>S<br>S           | SER | S<br>E |

\*position where Monte Carlo didn't find an alternative and where the top amino acid is the wild type

| $\infty$      |
|---------------|
| Į             |
| G.            |
| Ī             |
| $\mathcal{I}$ |
|               |

| 3.31     |
|----------|
|          |
|          |
| 'n       |
| LYS      |
| 214      |
| GLN      |
| 239      |
| GLU      |
| 109      |
| LEU      |
| 56       |
| ASP      |
|          |
| 380      |
| HSP<br>D |
| 170      |
| E<br>S   |
|          |

Table 7. Bndry4\_core4 - Monte Carlo Analysis (Ground State and Allowed Amino Acids and Their Number of Occurrences (For the Top 1000 Sequences)

\*position where Monte Carlo didn't find an alternative and where the top amino acid is the wild type

 $\perp$ 

FIG.\_9

Table 8. Bndrv4\_AD - Monte Carlo Analysis (Ground State and Allowed Amino Acids

| S  |                 |     |     |     |     |        |     |     |     |     |     |     |     |     | 62  |
|--|-----------------|-----|-----|-----|-----|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| no Acic  |                 |     |     |     |     |        |     |     |     |     |     |     |     |     | LYS |
| ed Ami<br>ices)  |                 |     |     |     |     |        | 123 |     |     |     |     |     |     |     | 209 |
| a Allow<br>Sequen  |                 |     |     |     |     |        | ΓYS |     |     |     |     |     |     |     | GLN |
| 1000 S   |                 |     |     |     |     | 277    | 217 |     |     |     |     |     |     |     | 230 |
| he Top   |                 |     |     |     |     | LYS    | GLU |     |     |     |     |     |     |     | GLU |
| s (For t   |                 |     |     |     |     | 223    | 225 |     |     |     | 321 |     |     |     | 136 |
| Ariarys<br>irrence   |                 |     |     |     |     | GLN    | HSP |     |     |     | ASP |     |     |     | LEU |
|  |                 | 112 |     |     |     | 89     | 133 | 394 | 213 |     | 305 |     |     |     | 22  |
| and Their Number of Occurrences (For the Top 1000 Sequences) |                 | LEU |     |     |     | H<br>H | Щ   | GLU | GLN |     | ALA |     |     |     | ASP |
| d Their N  | ם מ             | 887 | 666 | 984 | 931 | 357    | 287 | 605 | 982 | 962 | 373 | 926 | 994 | 666 | 304 |
| an<br>an   | Ground<br>State | Ш   | LEU | GLU | LE  | GLU    | VAL | GLN | ΓλS | GLU | HH  | LE  | LEU | ALA | HSP |
|  | Position        | 4   | 20  | 27  | 32  | 34     | 38  | 145 | 146 | 147 | 148 | 155 | 156 | 164 | 170 |
|  |                 |     |     |     |     |        |     |     |     |     |     |     |     |     |     |

\*position where Monte Carlo didn't find an alternative and where the top amino acid is the wild type

*≻ FIG.\_ 10* 

|   | 130  |   |            |
|---|--|---|------------|
|   | LYS  |   |            |
|   | 203  |   | 216        |
|   | GLU  |   | SLN<br>S   |
| 271   | 277  | 323   | 234        |
| E E   | HSP  | THR   | GLU        |
| 194   | 89<br>376<br>179   | 332   | 134        |
| LEU<br>VAL                                      | OCIC<br>OCIC<br>OCIC<br>OCIC<br>OCIC<br>OCIC<br>OCIC<br>OCIC       | ALA   | LEU        |
| 8 0 0 0 4<br>0 0 0 0 0 0<br>0 0 0 0 0 0         | 850<br>850<br>850<br>850<br>850<br>850<br>850<br>850<br>850<br>850 | 0 0 0<br>0 0 0 0<br>0 0 0 0 0 0 0 0 0 0 0 0 0 | 666<br>870 |
|   | GLN<br>GLN<br>LYS  | GLU<br>ASP<br>ILE<br>LEU                      | ALA<br>HSP |
| 4 0 2 7 8 8 4 8 4 8 9 4 8 9 9 9 9 9 9 9 9 9 9 9 | 38<br>145<br>146   | 147<br>155<br>156                             | 164<br>170 |
|   |  |   |            |

Table 9. Bndry4\_AD\_core4 - Monte Carlo Analysis (Ground State and Allowed Amino Acids and Their Number of Occurrences (For the Top 1000 Sequences)

Ground State

Position

\*position where Monte Carlo didn't find an alternative and where the top amino acid is the wild type

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ATGACTCCATTAGGTCCAGCTTCCTCTGCCGCAAAGCTTCCTGCTGAAACTGCTGGAACAGGTTCGTAAAATCCAGGGTGATGC <u> AGCTGCTCTGCAGGAAAAATCTGCGCTACCTACAAACTGTGCCATCCGGAAGÁACTGGTTCTGCTGGGTCACTCCCTGGGTATCC</u> CAGGGTCTGTTCCAGGCTTTCGAAGGTATCTCCCCGGAACTGGGTCCGACCCTGGACACTCTGCAGCTGGACGTCGCTGCTGACCTGG TACCACCATCTGGCAGCAGATGGAAGAACTGGGTATGGCTCCGGCTCTGCAGCCGACCCAGGGTGCTATGCCGGCTTTTCGCTTCCG CTITCCAGCGTCGCGCAGGTGGCATCCTGATCGCTAGCCACCTGCAGGCTTCCTGGAAGTTTCCTACCGTGTTTTCCGTCACCTG GCTCAGCCGTGA

## FIG.\_ 11A

ore4

atgactccattaggtccagcttcctctgccgcaaagcttcctgctgaaactgctggaactggaacaggttcgtaaaatccagggtgatgc **AGCTGCTCTGCAGGAAAACTGTGCGCTACCTACAAACTGTGCCATCCGGAAGAACTGGTTCTGCTGGGTCACTCCCTGGGTATCC** CAGGGTCTGCTGCAAGCTCTGGAAGGTATCTCCCCGGAACTGGGTCCGACCGTTGACACTCTGCAGCTGGACATCGCTGACCTGG TACCACCATCTGGCAGCAGATGGAAGAACTGGGTATGGCTCCGGCTCTGCAGCCGACCCAGGGTGCTATGCCGGCTTTCGCTTCCG CTTTCCAGCGTCGCCAGGTGGCATCCTGATCGCTAGCCACCTGCAGGCTTCCTGGAAGTTTCCTACCGTGTTTTCCGTCTCACCTG GCTCAGCCGTGA

## FIG. 11B

Core4v

**ATGACTCCATTAGGTCCAGCTTCCTCTCTGCCGCAAAGCTTCCTGCTGAAACTGCTGGAACAGATCCGTAAAATCCAGGGTGATGC AGCTGCTCTGCAGGAAAACTGTGCGCTACCTACAAACTGTGCCATCCGGAAGAACTGGTTCTGCTGGGTCACTCCCTGGGTATCC** CAGGGTCTGCTGCAAGCTCTGGAAGGTATCTCCCCGGAACTGGGTCCGACCCTGGACACTCTGCAGCTGGACATCGCTGACCTGGC TACCACCATCTGGCAGCAGATGGAAGAACTGGGTATGGCTCCGGCTCTGCAGCCGACCCAGGGTGCTATGCCGGCTTTCGCTTCCG CTTTCCAGCGTCGCGCAGGTGGCATCCTGATCGCTAGCCACATCCAGAGCTGGTTCGAAGTTTCCTACCGTGCTTTCCGTCGTCACCTG GCTCAGCCGTGA

FIG. 11C



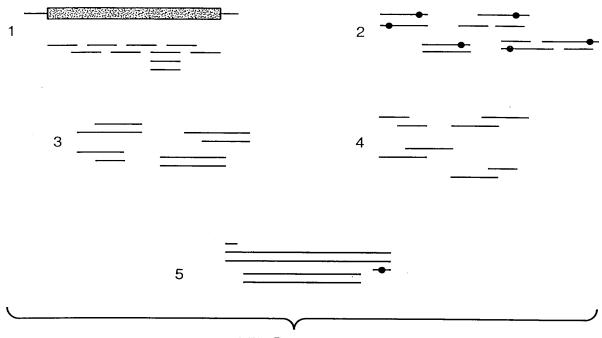


FIG.\_12

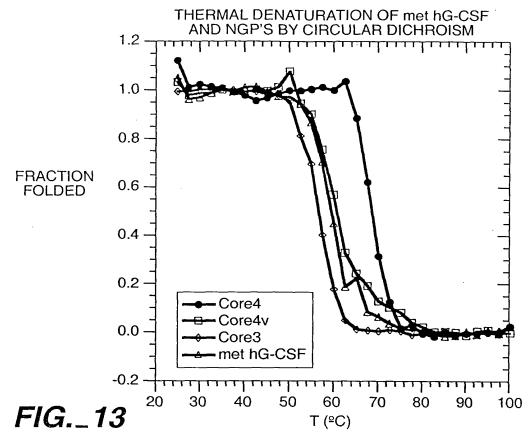
## -Melting Temperature (T<sub>m</sub>)

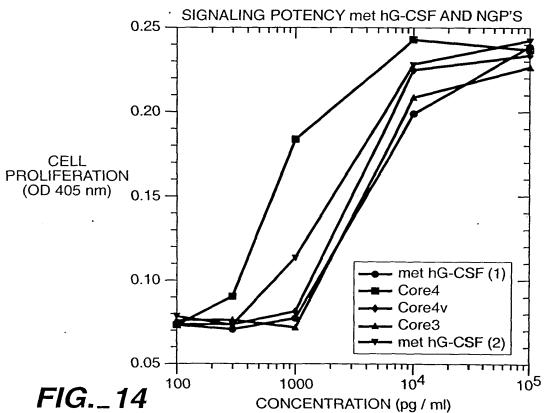
|          | T <sub>m</sub> (°C) | Extinction Coefficient (M <sup>-1</sup> cm <sup>-1</sup> ) |
|----------|---------------------|--|
| hG-CSFwt | 60                  | 15720  |
| core4    | 72                  | 14230  |
| core4v   | 61                  | 19730  |
| core3    | 58                  | 14230  |
| sm0*     | 63                  | 15720  |
| fm4*     | 63                  | 15720  |
| fm7*     | 70                  | 14230  |

\* Derived by reverting some core4 or core3 mutant positions to wild type

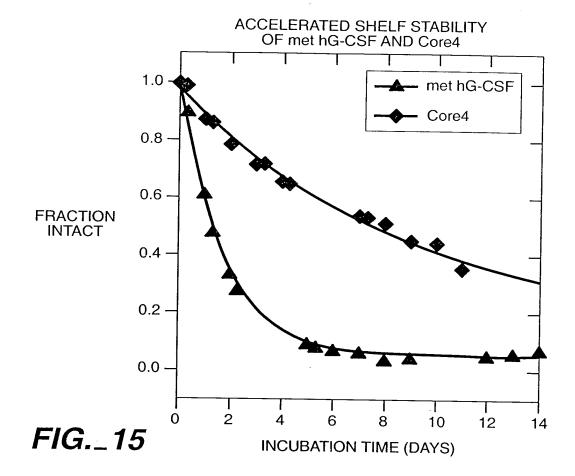
FIG.\_16







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